

# Concanavalin A Distinguishes Among Diseases of Altered Epidermal Differentiation

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**Mannose-containing glycoproteins from lesional tissue of several diseases of aberrant epidermal differentiation (palmar-plantar keratoderma, pachyonychia congenita, psoriasis, and epidermolytic hyperkeratosis) were analyzed by overlaying iodinated concanavalin A onto molecules separated by polyacrylamide gel electrophoresis. Gel autoradiograms showed that biopsy samples from patients with the same disease were very similar. The radioactivity profiles were different for each disease and were distinguishable from each other and from normal epidermis and callus. The resolution and sensitivity of this technique may be of diagnostic significance.**

Changes in cell surface glycoproteins occur with cellular differentiation [1,2]. Epidermal glycoproteins have been studied by reaction of frozen skin sections with fluorescein-conjugated lectins [3-5]. This approach can distinguish the binding of lectins with different sugar specificities to various strata of the skin [6-8]. Concanavalin A (Con-A) specifically labels the D-glucosyl and D-mannosyl residues of glycoproteins. Tissues reacted with Con-A were most intensely stained at the cell boundaries and intercellular junctions [4,9]. This was confirmed ultrastructurally with peroxidase-conjugated Con-A, where labeling was confined to the outer layers of the plasma membrane and the desmosomes [4,9]. Con-A has been reported to bind to all viable cell layers of the epidermis but not to the stratum corneum [5,7,8]. Cells at different stages of differentiation were not distinguishable by Con-A fluorescent labeling [8]. No difference was noted between normal and psoriatic lesional epidermis in this way [8].

Reaction of Con-A with tissue sections locates the lectin binding sites. It does not, however, identify the macromolecules responsible for the binding. The molecular weights of the glycoproteins comprising these binding sites may be obtained by an alternative experimental procedure. The proteins are first separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then the mannose-containing glycoproteins are tagged by overlay of the gel with [<sup>125</sup>I]Con-A. Using this technique with epidermal cells at different stages of differentiation, we have resolved by autoradiography some 20 glycoproteins and demonstrated major changes in the relative intensities of the labeled bands with the stage of differentiation [10].

In the present study, we compare the results for normal skin and for lesional tissue from 4 diseases of aberrant epidermal

differentiation. Palmar-plantar keratoderma of Unna Thost is a hyperkeratotic disease clinically resembling normal callus. Pachyonychia congenita exhibits acanthosis and an increased stratum corneum. Psoriasis involves abnormally high cell proliferation and turnover with a thickened spinous layer, attenuated granular layer, parakeratosis, and thickened sheets of stratum corneum. Epidermolytic hyperkeratosis is a hyperproliferative disease characterized by thickened spinous, granular, and horny layers; the granular layer is vacuolated and degenerated.

## MATERIALS AND METHODS

### Tissue

Superficial shave biopsies were taken from lesional tissue of patients (after informed consent). Biopsies of pachyonychia congenita were from the knees of 2 patients with this disease. Two epidermolytic hyperkeratosis samples were obtained from the arm and back of different patients. Psoriatic samples from 3 different patients included 1 from the arm and 2 from backs. A sample of plantar keratoderma was also used. Diagnoses were confirmed by routine histology after hematoxylin-eosin staining. For comparison, normal callus was obtained from plantar epidermis. Normal skin from the back was obtained from the Skin Bank at this University. It was separated into dermis and epidermis by heat treatment for 30 sec at 56°C; we have previously shown that this procedure does not alter the glycoproteins that react with [<sup>125</sup>I]Con-A [10].

### Polyacrylamide Gel Electrophoresis

Tissue samples were mixed at 4°C in Dulbecco's phosphate-buffered saline, pH 7.2, containing 2 mM phenylmethylsulfonylfluoride, homogenized in a ground-glass tissue grinder, then sonicated for 2 min in an ultrasonic cell disruptor. Aliquots of tissue samples were mixed with an equal volume of a buffer consisting of 0.125 M Tris-HCl (pH 6.8), 20% glycerol, 4% SDS, 10% 2-mercaptoethanol, 2 mM phenylmethylsulfonylfluoride, 0.004% bromophenol blue (all purchased from Sigma, St. Louis, Missouri). The mixture was heated at 100°C for 3 min. Equal amounts of cell protein, as determined by the Lowry assay [11], were loaded onto gel slots on 1.5-mm vertical slabs of 7.5% polyacrylamide below a 3% stacking gel. Proteins were separated by the system of Laemmli [12]. Electrophoresis was for 3 h at 30 mA per slab (at constant current). Gels were fixed for 2 h in methanol:acetic acid:water (5:1:5). Prior to reaction with lectin, the gels were reneutralized to pH 7.5 in many changes of Buffer A (50 mM Tris-HCl, 0.15 M NaCl, 0.1% NaN<sub>3</sub>, 0.5 mM CaCl<sub>2</sub>, 0.5 mM MnCl<sub>2</sub>). Molecular weight calibration was achieved with the following standards: myosin (200K), phosphorylase B (92.5K), bovine serum albumin (68K), ovalbumin (46K), and carbonic anhydrase (30K).

### Lectin Iodination and Autoradiography

Con-A (purchased from Sigma) was iodinated by the procedure of Burridge [13]. It was dissolved in 200 microliters (μl) of Buffer A, at a concentration of 25 mg/ml, together with α-methyl-D-mannoside at 40 mg/ml (Sigma). To the reaction mixture were added 1 mCi of Na [<sup>125</sup>I] (Amersham, Arlington Heights, Illinois) and 10 μl of chloramine T at 5 mg/ml (Sigma). After iodination for 30 min at room temperature, the reaction was terminated by the addition of 10 mg/ml of sodium metabisulfate. The iodinated lectin was separated from the inhibitory sugar by passage through a small column of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, California). The lectin was eluted with Buffer A, then dialyzed for 24 h against Buffer A at 4°C. The specific activity of the iodinated Con-A was approximately 10<sup>7</sup>-10<sup>8</sup> cpm/mg. SDS-PAGE gels were overlaid with [<sup>125</sup>I]Con-A (10<sup>7</sup> cpm/ml) in Buffer A containing

Manuscript received June 7, 1983; accepted for publication August 10, 1983.

This work was supported by Grant CA 27441 awarded by the National Cancer Institute, and Grant H-869 awarded by the Robert A. Welch Foundation.

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#### Abbreviations:

Con-A: concanavalin A

SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2 mg/ml of bovine hemoglobin (Sigma) as a carrier. After reaction for 2 h at room temperature in a moisture-filled box, the gels were washed for 3–4 days in many changes of Buffer A, then dried in a Bio-Rad gel dryer on Whatman 3MM paper. The specificity of lectin binding was tested with controls in which the inhibitory sugar for Con-A ( $\alpha$ -methyl-D-mannoside) at a 4% concentration was dissolved in Buffer A, and the modified buffer was used when washing and overlaying the gels with [ $^{125}$ I]Con-A. Sheets of Kodak SB-5 film were exposed to the gels for several days. The autoradiograms were scanned with a Quick Scan densitometer (Helena Laboratories, Beaumont, Texas).

## RESULTS

Fig 1 displays autoradiograms from SDS-PAGE gels that were overlaid with [ $^{125}$ I]Con-A. In a control experiment (not illustrated), inclusion of the Con-A inhibitory sugar  $\alpha$ -methyl-D-mannoside together with the iodinated lectin completely obliterated the uptake of radioactivity from [ $^{125}$ I]Con-A. Autoradiograms of samples from different patients with the same disease are grouped together in Fig 1. Samples of normal epidermis and callus from different subjects are also compared.

To facilitate quantitative comparisons, densitometer scans of the autoradiogram of one gel are presented in Fig 2. The radioactivity of the dermis (Fig 2a) peaks at 150K and is minimal below 80K, whereas the epidermis is predominantly labeled at lower molecular weights. The major bands for the normal epidermis (Fig 2b) are near 40K and 75K, with several lesser bands in between. The radioactive labeling for normal plantar callus (Fig 2c) is generally much weaker than that for the epidermis, with peaks again at 40K and (attenuated) at 75K. For plantar keratoderma (Fig 2d), the radioactive profile resembles that of normal callus except for the addition of a more intense band at 50K. The major band for pachyonychia congenita (Fig 2e) is at 40K; at the next level of intensity are labeled bands at 80K and 50K. The strongest radioactivity for psoriasis (Fig 2f) is an unresolved doublet at 80K and 75K; a less intense peak occurs at 50K. For epidermolytic hyperkeratosis (Fig 2g), an extremely radioactive band at 50K dominates the profile; the other peaks are relatively low.

## DISCUSSION

We have previously found some 20 [ $^{125}$ I]Con-A reactive glycoproteins in normal epidermal cells. Their relative labeling intensities on SDS-PAGE gels changed dramatically with the stage of differentiation [10]. The present study was designed to explore similar differences in aberrant differentiation as expressed in a number of epidermal diseases. Con-A has been shown to bind primarily to epidermal cell boundaries and

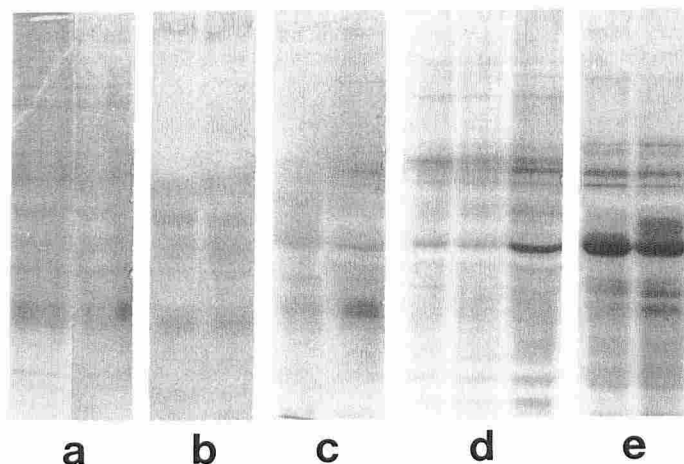


FIG 1. Autoradiograms of SDS-PAGE gels overlaid with [ $^{125}$ I]Con-A. Each lane corresponds to a biopsy specimen from a different subject. To test reproducibility, they are grouped by diagnosis of the tissue as (a) normal epidermis, (b) normal callus, (c) pachyonychia congenita, (d) psoriasis, (e) epidermolytic hyperkeratosis.

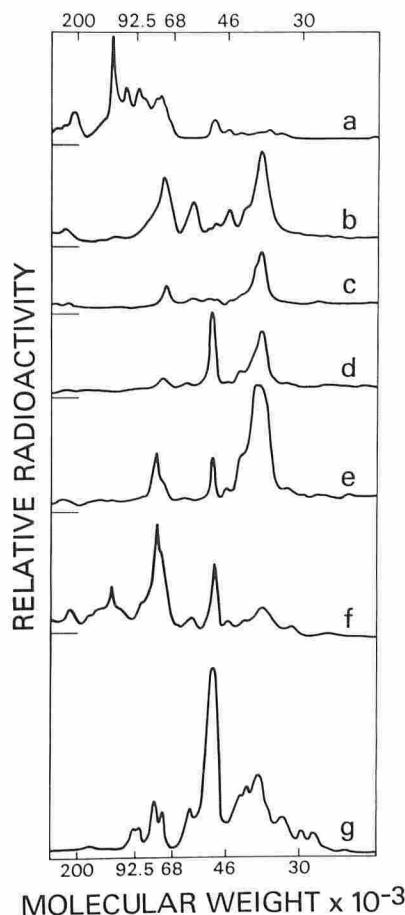


FIG 2. Densitometer scans of the autoradiogram of an SDS-PAGE gel overlaid with [ $^{125}$ I]Con-A: (a) normal dermis, (b) normal epidermis, (c) normal callus, (d) palmar-plantar keratoderma of Unna Thost, (e) pachyonychia congenita, (f) psoriasis, (g) epidermolytic hyperkeratosis.

intercellular junctions [4,9]. The variation in Con-A binding for different diseases suggests that cell surface changes are associated with aberrant as well as normal differentiation.

Fig 1 illustrates the reproducibility of the results. Samples of normal epidermis from different subjects resemble each other, as do samples of callus. Samples from different patients with the same disease (sometimes from different parts of the body) also yield remarkably similar patterns, apart from some intensity variations.

During SDS-PAGE, each gel slot was loaded with the same amount of protein (as determined by the Lowry assay). This does not insure a precise equality in the amount of glycoprotein present in each slot, but it can be expected to yield roughly comparable quantities. The scans in Fig 2 were taken at a fixed setting from the autoradiogram of a single gel. The relative amplitudes of the curves thus reflect, at least qualitatively, the comparative specific activities of the peaks.

The tissue from the Skin Bank was heat-separated into dermis and epidermis. The other samples were small superficial shave biopsies for which this could not be done. The issue of possible dermal contamination of the latter needs, therefore, to be resolved. Fig 2a shows that the radioactivity in the dermis is concentrated at higher molecular weights and falls off sharply below 80K. Conversely, the labeling pattern of the normal epidermis (Fig 2b) extends from a peak at 75K downward, with no substantial radioactivity at the higher molecular weights. This fortunate lack of overlap provides us with a sensitive criterion of dermal contamination for the other epidermal samples. It can be seen (Fig 2c–g) that this is not a problem as there is little or no labeling above 80K.

The radioactivity profiles for the 4 diseases are easily distinguished from each other and from normal skin (epidermis or callus). The pattern recognition can be simply expressed in terms of the 3 most conspicuous features: bands at 40K and 50K and a doublet at 75K and 80K (which is mostly poorly resolved and will be treated as a single structure). All the disease samples have an intense band at 50K whose counterpart is barely discernible for normal skin.

For the normal epidermis (Fig 2b) the 40K peak is clearly the most intense, the 75K peak is next, and the other features are substantially weaker. The radioactivity uptake of the callus (Fig 2c) is considerably less than that of the normal epidermis, with only the 40K band still appreciable. The callus is characterized by a thickened stratum corneum. In experiments with fluorescein-conjugated Con-A [5,7,8], no labeling of the stratum corneum was observed.

Keratoderma clinically resembles normal callus. Its radioactive profile (Fig 2d) is also very similar to that of callus (Fig 2c) with the exception of the addition of the 50K band.

While the stratum corneum is thickened in pachyonychia congenita, the Con-A profile (Fig 2e) does not resemble that for callus (Fig 2c) but is instead qualitatively more similar to that of normal epidermis (Fig 2b). As for normal epidermis, the dominant band is near 40K and the second most prominent feature is the 75–80K doublet. The most conspicuous difference is the occurrence in the diseased sample of a 50K peak comparable with the 80K peak.

The most striking feature of the Con-A profile for psoriasis (Fig 2f) is the near disappearance of the 40K band. The most intense peak is at 80K with the 50K peak next in prominence.

For epidermolytic hyperkeratosis (Fig 2g), the Con-A profile is dominated by an extremely intense peak at 50K, with all the other features greatly reduced.

Some speculations are suggested by a correlation of the results. The 50K glycoprotein appears to be a marker of aberrant differentiation. It is barely discernible for normal epidermis or callus, but is prominent for all disease samples. It is tempting to associate the 40K glycoprotein with the granular layer. While it is present for all samples, it is strongly attenuated in psoriasis for which the granular layer is absent) and is also reduced in epidermolytic hyperkeratosis for which there is degeneration of the granular layer). In fact, the 40K band was not much in evidence in cultured keratinocytes in our earlier study [10]; these cells do not form a granular layer in the culture system.

We have shown that an empirical pattern recognition of Con-A-reactive glycoproteins can be used to distinguish among diseases of altered epidermal differentiation. Knowing how to ascribe functional roles to the principal macromolecules observed would contribute to understanding the nature of the

molecular lesion involved in these diseases. This ultimate aim cannot be fully achieved without the availability of specific antibodies to these glycoproteins, which in turn await their isolation and biochemical characterization. At this point, we cannot elucidate the role of any particular glycoprotein in the etiology of the diseases, nor do we assert that mannose-containing glycoproteins (which bind Con-A) are more or less important than other glycoproteins. We simply observe that Con-A iodination patterns corresponding to lesional samples of different diseases are distinguishable.

The use of SDS-PAGE in conjunction with [<sup>125</sup>I]Con-A thus provides the resolution and sensitivity to identify a distinct glycoprotein pattern for each disease from small shave biopsies of lesional tissue. This technique might prove to be a practical diagnostic tool. By contrast, biochemical extractions and lectin affinity chromatography require much greater quantities of tissue which cannot reasonably be taken from human subjects in clinical practice.

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